

Supporting Information

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Anti-cholinesterase Activities of Hydrolysable Tannins and Polyhydroxytriterpenoid Derivatives from *Terminalia chebula* Retz. Fruit

Min-Soo Kim ^{1,2}, Dong Young Lee ³, Sang Hyun Sung ³

and Won Kyung Jeon ^{1,2 *}

¹ Herbal Medicine Research Division, Korea Institute of Oriental Medicine, Daejeon 34054, South Korea

² Convergence Research Center for Diagnosis, Treatment and Care System of Dementia, Korea Institute of Science and Technology, Seoul 02792, South Korea

³ College of Pharmacy, Seoul National University, Seoul 08826, South Korea

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* Corresponding author: E- Mail: wkjeon@kiom.re.kr (W.K. Jeon), Phone +82-42-868-9505, Fax: +82-42-868-9541.

S1. Reagents

AChE and BChE activity assay kits were purchased from BioVision (BioVision Co., Milpitas, CA, USA). All solvents required for extraction and isolation were purchased from Daejung chemicals (Daejung, Si-heung, South Korea). The other reagents, including dimethyl sulfoxide (DMSO), donepezil, and galantamine, were purchased from Sigma-Aldrich (Sigma, St. Louis, MO, USA).

S2. AChE and BChE inhibitory activity assay

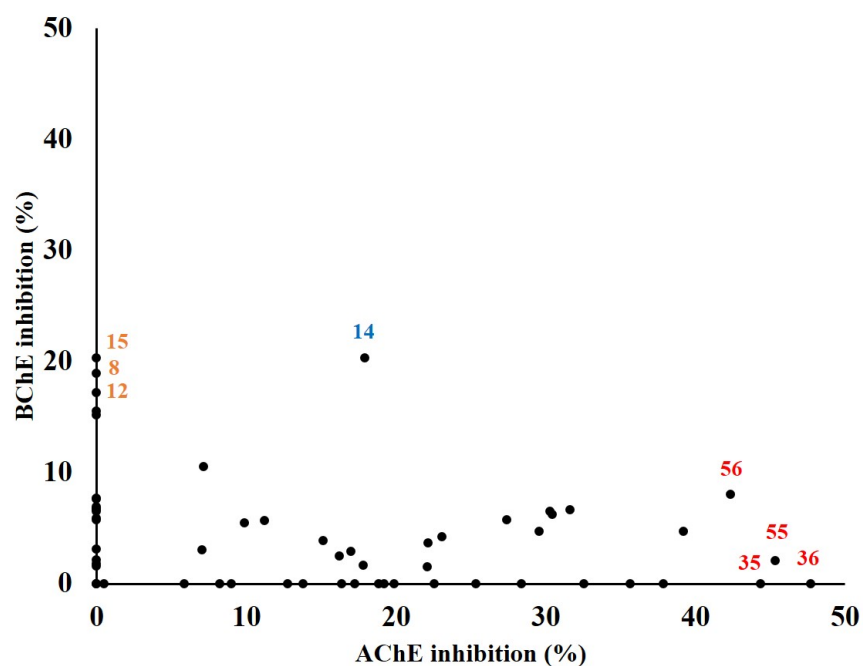
AChE and BChE inhibitory activities were determined using a colourimetric microplate assay kit according to the manufacturer's instructions, which were based on Ellman's method [1]. Briefly, each sample was dissolved in DMSO and diluted in distilled water. Ninety-five microlitres of prepared sample, 5 μ L of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and 10 μ L of enzyme in assay buffer were added to a 96-well microplate. After shaking incubation for 10 min at room temperature in the dark, 100 μ L of substrate was added. The change in absorbance was monitored at 412 nm every 10 min with a microplate reader (BioTek, Winooski, VT, USA). The percentage inhibition of ChE was calculated using the following formula:

$$\text{AChE activity Inhibition (\%)} = 1 - \frac{As - As'}{Ac - Ac'} \times 100$$

where A_s is the absorbance of the sample; A_s' is the absorbance of the sample without enzyme; A_c is the absorbance of 0.1 M phosphate buffer (pH 8.0) instead of sample; A_c' is the absorbance of 0.1 M phosphate buffer without enzyme. Donepezil and galantamine were used as positive controls.

S3. Data analysis

The concentration of each TCE compound that inhibited AChE activity by 50% (IC_{50}) was calculated using a graph with dose-inhibition curve by regression analysis with at least five appropriate concentrations. The assays were done in triplicate. All data are expressed as mean \pm SD (Standard deviation).



S4. Figure S1. Distribution of ChE inhibitory activities of 60 compounds from *T. chebula*. Scatter plots illustrate AChE and BChE inhibition by compounds. The ID numbers of compounds that exhibit strong inhibition of AChE or BChE are displayed.

References

- [1] G.L. Ellman, K.D. Courtney, V. Andres and R.M. Featherstone (1961). A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol.* 7, 88-95.